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Supplementary Materials for Article

Effects of Mindfulness-Based Therapy on Clinical Symptoms and DNA Methylation in Patients with Polycystic Ovary Syndrome and High Metabolic Risk

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Abstract: Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disorder affecting women of reproductive age. Research has shown that epigenetic alterations such as DNA methylation may play a role in the development and progression of abnormal ovarian function and metabolic disorders in PCOS. Among other epigenetic changes, studies have identified specific genes (related with insulin signalling and steroid hormone metabolism) that are methylated in women with PCOS. DNA methylation appears to respond to various interventions aimed at altering health and lifestyle factors. We tested the efficacy of a mindfulness-based stress reduction program (MBSR) in PCOS patients. We examined its effects on anthropometric measurements, mental health and wellbeing, and alterations in DNA methylation in peripheral blood. MBSR was associated with a reduction in body mass index, waist circumference and blood glucose level, an improvement in subjectively perceived general health, emotional role limitation, and levels of pain, as well as mindfulness-like traits. MBSR reduced the expression of anxious symptomatology and subjectively perceived stress. Methylation changes were observed in four genes: COMT, FST, FKBP51, and MAOA. We conclude that MBSR may be a useful supplementary therapy to mitigate the deleterious effects of PCOS on mental health.

Keywords: mindfulness-based stress reduction program, polycystic ovary syndrome, epigenetics, DNA methylation, candidate genes, depression, anxiety

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Table S1. Amplicon primer sequences and annealing temperatures for candidate genes. Illumina universal sequences are presented in grey shade and are followed by the sequence-specific primers that align on the target bisulfite converted DNA sequence.

Amplicon	Forward or reverse primer	Primer Sequence	Annealing temperature / °C
SLC6A4_3	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGTTAAAGAGTAGGAAAGTTAGGATTTT	54.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ACCCTCACATAATCTAATCTCTAAA	
SLC6A4_5	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTTGAGTAGTTGGGAATATAAG	52.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AAAACCCTTATCTAATTCCTCTC	
COMT_1[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GTTTTTAATTTTGTATAGGTAAGAT	54.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TACCCTCCCTACCCACAAC	
COMT_2[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGTTATTTGTGGTTAGAAGTAGTT	54.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ACTACCCCAAAAACCCCAC	
COMT_4[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNN**TGTAGGAGGAGTATAGAGTATTGG	62.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNN**TCATAACCCACTCCTTCTACT	
BDNF_81_1[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TGAGGGTAGGTAAAGGGTAGT	55.3
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CAACTCTCCCAAAAAACCCTAC	
BDNF_81_2[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTATATAGGTTTTTGTGGGTAATTAG	55.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AAAAAAAAAAAAACTTCTTAAAAAAAT	
BDNF_81_3[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTTTTAGTTATGATGGGGGAGG	58.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CAAATCACACCTAAAACTCC	
BDNF_14_1[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GAGTTTATTTAGTATTTTGGATAGA	55.3
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AAAAATCTATTCCAACCTACACC	
BDNF_58_1[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTTTTTAAGGGAAGGGGAGTT**	54.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAACTAAAAATATTCTTCTCCACC**	
BDNF_58_2[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATAGAGTTATTAATTAGTTGGA**	55.0

	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-TAAATCCCTAAACTCCCTAAAA**	
BDNF_95_1[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTTTTAATGAGATATTTAT**	58.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAAATCCCCCAATCAACTCTCT**	
BDNF_95_2[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AAATGTTGTTATTATTTTGATTGAATT	54.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AACACCCAAATTCTCTAAAAAA	
BDNF_95_5[a]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNN**TTTTTTAGAGAATTTGGGTGT	56.7
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNN**AAACCTATCCTCACCTCCT	
TPH2_1[b]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGGTTTGTAATTTGATTGTGGT	52.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CCAATATTTACTAAAAAACATCATCA	
HTR1A_1[b]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTTTAGATATTTTTGGGATTGGAGAT	55.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-**CAAAAACCCAAACAAAAAATTCTTAC	
HTR1A_2[b]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-**TGGTTTATTGGTTTTTTTATTTTTAT	50.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-CAG**CTCCATTCACACTCTTCTTAAAC	
MAOA_1[b]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATAGTTTTTAGTTAAAATTAAAGAATGAA	50.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-**TACCCACTCTTAAAAACCAACC	
MAOA_2[b]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGGAGTTGATAGAAGGGTTTTTT	59.0
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CAAACCATAACTACACTACACTCTC	
CEBPB_1	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TGGTTTGGGATTTAGTATGTT	52.1
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TTCTTACAATTCTTACCCCC	
CEBPB_2	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATTGGGAAGGGGATTTAT	50.2
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ACCACTTCCATAAATTTAAAAACA	
EPHX1_2	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TGTAGAGGGATTTGTAGTTGGTT	55.3
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ACTTAAATAACAAATTAAATACATTTC	

EPM2A_1	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTGGGGTGGTGGTGTTAT	55.3
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATACCTTCCCAAAAAAACTCTCC	
FKBP51_F1[c]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGGGGGTGTTAGTTTTTATTATTTTTT	50.2
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ACTCCGCTAACCCTTCAAC	
FST_1	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGGATAGAAATTGGGGAGTTTT	55.3
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TACCTAACTTTACAACCACCCC	
FST_2	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTTGAAGTGGGTGTTTTTTTT	50.2
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATCAAATAACATTTCCCACCTT	
FST_3	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGAAGGTGGGAAATGTTATTTG	55.3
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AACAACAAAAAACAAAACCCAC	
IGFBP1_1	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGTTTGTGTTTTTTATAAGGTG	55.3
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATCAACAAAAACAATACCAACCA	
IGFBP1_2	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGTTGGTATTGTTTTTGTTGA	50.2
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CAAAAATTACTACTCCCCC	
INSR_1	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATGGGGAGAGGATTTTATTTA	50.2
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AACCCAATAACCCCACTTC	
LHCGR_1	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGTAGGAAGGAGGTTATTGGGTTAT	56.7
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CTACAACAACAACAACAACTTCAAC	
NR3C1_1[c]	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TTTTTGAAGTTTTTTTAGAGGG	55.3
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**AATTTCTCCAATTTCTTTTCTC	
PPRG1A_1	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**TAGGATTGTGTGTGGAGTTGTT	52.1
	Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CCAACTACCCAAAAAAAACACTC	
TBKBP1_1	Forward primer	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GAAGGTATTTATTGATTGA	52.1

Reverse primer	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGA-CAG**AATTCCCAAAACTATCTC

[a]Primer design according to Kouter et al. [99].
[b]Primer design according to Kouter et al. [60].
[c]Primer design according to Yehuda et al. [63].

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